

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number:

0 355 849
A2

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 89115739.8

(51) Int. Cl. 4: **G01N 21/64** , **G01N 35/02** ,
G01N 33/543

(22) Date of filing: 25.08.89

(30) Priority: 26.08.88 JP 210741/88

(43) Date of publication of application:
28.02.90 Bulletin 90/09

(84) Designated Contracting States:
DE FR GB

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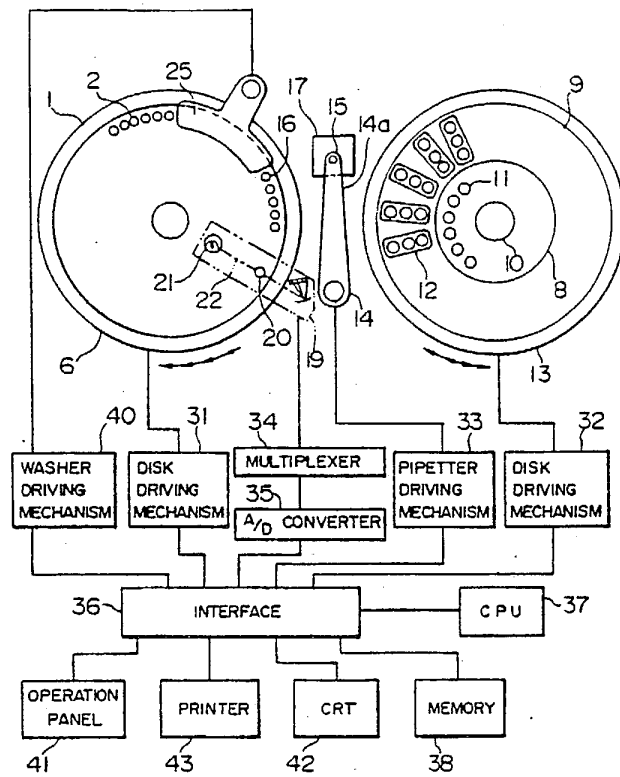
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(54) **Method and apparatus for automatic measurement of fluorescence.**

(57) An analyzer has a reaction disk (1) for holding a plurality of reaction containers (2) and a fluorophotometer (19) for measuring fluorescence stemming from solutions in the containers. Most of the containers contain solid phases (50) attached with antibodies (51) but at least one container does not contain any solid phase. In normal operation of the analyzer, a test sample (11) containing antigens (53) and a latently fluorescent reagent such as antibodies labeled by enzyme are added to a container (2) containing a solid phase (50). In this container, a fluorescent substance is created through an enzyme reaction. Exciting light (22) is irradiated on the container and fluorescence emitted from the fluorescent substance is measured. In mid course of measurement operation of test samples, fluorescence stemming from a reference sample (12) such as quinine sulfate is measured to produce measured values for the reference sample by which measured values for the test samples are corrected. Prior to measurement of general test samples (11) by the analyzer, fluorescence stemming from a reference sample (12) in a container removed of any solid phase is measured to enable the analyzer itself to decide whether the analyzer operates normally. In this case, a value measured for the reference sample is compared with a setting value. When the results of comparison indicate that the normal operation is difficult to continue, the sampling operation by the pipetter is discontinued.

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FIG. 2



METHOD AND APPARATUS FOR AUTOMATIC MEASUREMENT OF FLUORESCENCE

BACKGROUND OF THE INVENTION

This invention relates to method and apparatus for automatic measurement of fluorescence and more particularly to analytical method and apparatus applicable to measurement of a fluorescent substance created by adding a latently fluorescent reagent to a sample to be tested.

In the past, a radio-immunoassay method using radioisotopes has been employed widely in order to measure, on the immunologic base, a small amount of substances in a biological sample but recently a measurement method using enzyme or liposome has been predominant.

An enzyme immunoassay method is disclosed in, for example, JP-A-62-50662. In this example, a complex of antigen, antibody and enzyme is created on the surface of a bead and the intensity of fluorescence caused by an enzyme reaction, which develops when a substrate is brought into contact with the complex, is measured.

The above JP-A-62-50662 uses an ordinary fluorophotometer to measure reaction solutions and fails to refer to an apparatus using an automated reaction system and a fluorophotometer in combination.

On the other hand, in the field of automatic chemical analyzer, the analyzer itself changes with time so that measured values are affected by such a drift change and therefore countermeasures against this problem have been contrived. For example, U.S. Patent No. 4,043,756 discloses photometry of light absorbed by reaction solutions of samples to be tested which are sucked in flow cells. Before carrying out measurement of test samples, a reaction solution of a reference sample and a reagent blank are first measured with a view of correcting a standard curve, and a drift variation of the analyzer per se is corrected using a corrected standard curve.

When designing an immunoanalyzer by incorporating a fluorophotometer into an automatic analyzer, it is of significance to ensure high stability of the detection system and high reliability of results of measurement in a practiced immunoanalyzer. In the immunity measurement, quantitative measurement of a small amount of antigens or antibodies in a biological sample is needed and abnormality in performance of the optical system for fluorophotometry leads to impairment of analysis of small amounts. Further, degradation of reagent is a factor of increasing measurement error in the analysis of small amounts.

The aforementioned U.S. Patent No. 4,043,756 described drift variations of the analyzer but does not consider the performance degradation of the optical system and the reagent degradation. A reference sample used in this literature is a reaction solution prepared by reacting the same element as a target element in a test sample in the same way. In immunoanalysis, however, it is difficult to obtain a chemically stable solution of a substance which may be used as a reference sample in reactions.

SUMMARY OF THE INVENTION

An object of this invention is to provide method and apparatus which can decide automatically whether an analyzer capable of measuring fluorescence is maintained at proper performance.

Another object of this invention is to provide method and apparatus which can produce highly accurate results of fluorophotometry of a target element in a sample to be tested.

Still another object of this invention is to provide method and apparatus which can improve stability of measurement when quantitative measurement of concentration of antigens or antibodies in a test sample is effected indirectly by measuring fluorescence stemming from a fluorescent substance in a reaction solution which results from immunoreaction.

According to the invention, fluorescent substances are manifested corresponding to the amount of target elements in a test sample contained in a reaction container, and the intensity of fluorescence stemming from a reaction solution in the reaction container is measured by means of a fluorophotometer.

This can be done through the steps of measuring the intensity of fluorescence from a reference sample which can fluorescence, comparing a predetermined setting value with a measured value of the reference sample, and discontinuing the subsequent sampling operation by pipetting means when the results of comparison indicate that the measured value is smaller than the setting value.

When the results of comparison indicate that the measured value is larger than the setting value, the subsequent sampling operation by the pipetting means is allowed to continue and thereafter a reaction processing and a fluorophotometry processing for general test samples are carried out sequentially.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a flow chart for explaining the operation of an embodiment of the invention.

Fig. 2 is a schematic plan view illustrating an immunoanalysis apparatus for realizing the embodiment
5 operable in accordance with the Fig. 1 flow chart.

Fig. 3 is a graph showing an example of a standard curve for T_4 (thyroxine).

Fig. 4 is a graph showing an example of a standard curve for TSH (thyroid stimulating hormon).

Fig. 5 is a schematic representation useful for explaining the reaction in a fourth embodiment of the
invention.

Fig. 6 is a graph showing an example of a standard curve for TSH used in the fourth embodiment.
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DESCRIPTION OF THE PREFERRED EMBODIMENTS

In an immunoanalysis apparatus to which the invention is applied the lower limit of magnitude of
15 measured signals necessary for the analysis of small amounts is precedently set through an input unit and
stored in a memory unit. Used as a fluorescent reference sample is a solution of a chemically stable
substance such as for example quinine sulfate. A sample to be tested (general test sample) and the
reference sample are pipetted into corresponding reaction containers in a movable train of reaction
containers. While a latently fluorescent reagent is added to the reaction container for test sample, no
20 latently fluorescent reagent is added to the reaction container for fluorescent reference sample so that
fluorescence stemming from the solution itself may be measured. In this manner, the intensity of
fluorescence which is substantially constant can be obtained reiteratively as far as the performance of the
optical system remains constant.

Prior to fluorophotometry of reaction solutions based on general test samples, the intensity of
25 fluorescence from the reference sample is measured and a measured value is compared with a setting
value stored precedently in a memory unit. When the intensity of fluorescence from the reference sample is
below the setting value, indicating that the optical system is placed in a state which does not meet the
condition for the analysis of small amounts, the subsequent pipetting of the test sample by means of a
pipetter is discontinued to prevent invalid measurement. In such an event, the operator checks the light
30 source and photoelectric detectors and replaces degraded components with new ones, as necessary, to
enable the optical system to recover necessary functions. After recovery of the function of the optical
system, the pipetter is allowed to start sampling the general samples and a reaction disk is also allowed to
start normal operation. Subsequently, test samples which have already been contained in several reaction
containers on the reaction disk may be brought into the analysis processing under programmed control
35 without being discarded.

When results of comparison between the measured value for the reference sample and the setting
value indicate that the intensity of fluorescence stemming from the reference sample exceeds the setting
value, indicating that the optical system is placed in a satisfactory state, the subsequent pipetting of test
samples is allowed to continue.

40 In a preferred embodiment of the invention, the upper limit of values of fluorophotometry of a reagent
blank is set precedently and stored. When the reagent blank value measured midway of analysis of a
plurality of test samples exceeds the upper limit, an alarm indicative of degradation of reagent is delivered,
thus urging the operator to exchange the reagent with new one.

An immunoanalysis apparatus to which the invention is applied may be constructed as shown in Fig. 2.

45 Referring to Fig. 2, a plurality of reaction containers 2 are held in array on a rotary circular reaction disk
1 along its circumferential edge and the reaction disk 1 is rotated intermittently by means of a disk driving
mechanism 31. The reaction container 2 suitable for fluorophotometry is made of a transparent material
such as glass or acryl resin. A fluorophotometer 19 has a photometric position 20 which lies inside a
reaction thermostatic tank 6.

50 The photometer 19 is of a multi-wavelength photometric type having a plurality of detectors and it faces
the reaction container 2 so that when a reaction container 2 is at the photometric position 20 on the reaction
disk 1, flux of light 22 emitted from as light source lamp 21 transmits through that reaction container 2.
Exciting light, monochromatic light of a predetermined wavelength, is irradiated on the solution in the
reaction container from above or below the reaction container, and fluorescence emitted from the solution
55 transmits through the side surface of the container and monochromatic light of a predetermined wavelength
is selected and detected by a photomultiplier tube serving as the photoelectric detector.

On the other hand, a plurality of reagent cups 12 needed for a plurality of kinds of analysis items are
held in array on a reagent disk 9 which is rotatable clockwise or counterclockwise. Each of the reagent cups

12 has a plurality of (for example, three) reagent solution storage chambers and individual chambers store a first reagent, a second reagent and as necessary a third reagent which correspond to specified analysis items, respectively.

The reagent disk 9 and a sample disk 8 are rotated about a center shaft 10 by a number of pitches designated by a controller and this rotational operation is accomplished by a disk driving mechanism 32. With the above construction, a sample container 11 containing a target test sample and a required reagent cup 12 on the reagent disk 9 can be positioned and stopped at positions where the requisite test sample and reagent are sucked. Sample cups 11 are maintained at a predetermined temperature inside an air thermoconstant tank. The reagent cups 12 are maintained at a low temperature of 10°C or less by means of a cooling tank 13.

The reference sample or reference solution is formed of a liquid or solution containing a substance which is able to be excited to fluorescence by itself and for example, may be quinine sulfate, rhodamine B or water. In this embodiment, one of the reagent cups 12 contains a quinine sulfate solution at a predetermined concentration.

An arm 14a can be moved vertically (orthogonally to the sheet of drawing) and rotated about a shaft 14 by means of a pipetter driving mechanism 33. The arm 14a is mounted with a liquid charge/discharge nozzle or pipetting probe 15 which communicates with a cylinder in a pipetter driving mechanism 33 through a liquid flow path. The probe 15 can be moved vertically at any one of the sample suction position on the sample disk 8, reagent suction position on the reagent disk 9, liquid discharge position 16 on the reaction disk 1 and probe washing position 17. During the first cycle of rotation of the reaction disk 1, the probe 15 may be operated to pipette a predetermined amount of sample from a sample cup 11 to a reaction container at a discharge position 16 and during the second cycle of rotation of the reaction disk 1, the probe 15 may be operated to pipette a predetermined amount of reagent from a reagent cup 12 to the reaction container at that discharge position 16.

In an alternative, the probe 15 may be operated to pipette a test sample and a first reagent during the first cycle of rotation of the reaction disk and to pipette a second reagent and as necessary a third reagent during the second and ensuing cycle of rotation of the reaction disk.

When proceeding with an analysis operation of a general test sample such as a blood serum, solid phases attached with antibodies (or antigens) corresponding to individual analysis items i.e., kinds of antigens or antibodies are stored precedently in reaction containers. For example, if a bead is used as solid phase, the surface of the bead is coated in advance with, for example, specified antibodies.

When a reaction container 2 storing a solid phase attached with antibodies reaches a discharge position 16, a sample sucked into the probe 15 from a sample cup 11 is discharged into that reaction container. After discharging, the interior and exterior of the probe 15 are washed by means of a washing unit also designated by reference numeral 17. Subsequently, the probe 15 sucks a reagent solution for immunoreaction from a selected reagent cup 12 and adds the reagent to the same reaction container 2 still staying at that discharge position 16. The immunoreaction reagent solution contains antibodies labeled by enzyme. Thereafter, the reaction disk 1 is advanced clockwise by one step. Similar sampling operations are sequentially undertaken by the probe 15, serving as a pipetting mechanism, for the following reaction containers.

In the reaction container containing the test sample added with the immunoreaction reagent (that is, a first reagent), an antigen standing for a target element in the sample couples to an antibody attached to the bead and the antibody labeled by enzyme couples to the coupled antigen. Thus, immunocomplexes are created on the bead through antigen/antibody reaction. When the reaction container containing the immunocomplex reaches a region of a washer 25 having a plurality of nozzles, a liquid containing part of the reagent which has not been reacted is discharged from the reaction container and accordingly the bead having the immunocomplex remains in the reaction container. In the next step, a cleansing solution is charged into the reaction container, and the cleansing solution is then discharged after cleansing, leaving the bead having the immunocomplex in the reaction container. The above bead washing operation is repeated plural times. The washer 25 is operated for vertical movement and charging/discharging by means of a washer driving mechanism 40.

When the reaction disk 1 is further advanced and the reaction container 2 containing the washed bead reached a discharge position 16, the probe 15 is operated to suck a latently fluorescent reagent standing for a second reagent from a specified reagent cup 12 and add the second reagent to the bead. As the latently fluorescent reagent, a solution may be used containing a substrate which is able to turn into a fluorescent substance. In the reaction container 2, the substrate reacts with the enzyme attached to the solid phase so as to turn into the fluorescent substance.

When the reaction disk 1 is further advanced stepwise and the reaction container 2 containing the

fluorescent substance reaches the photometry position 20, exciting light is irradiated on the container by means of the fluorophotometer 19 to measure the intensity of fluorescence stemming from the reaction solution in the container. Output signals produced from a plurality of photodetectors of the photometer 19 are applied to a multiplexer 34 at which only a signal based on requisite monochromatic light is selected. The selected signal is converted by an analog/digital converter 35 into a digital signal which in turn is stored into a memory unit 38 such as a random access memory or a floppy disk through an interface 36 under the control of a central processing unit (CPU) 37.

Prior to carrying out a series of analysis operations described previously for a blood sample standing for a general test sample with the analyzer shown in Fig. 2, the lower limit for deciding the performance of the optical system and the upper limit for deciding degradation of the reagent are set in the analyzer. A setting value for deciding the performance of the optical system and an acceptable upper limit value for deciding the reagent degradation are inputted by the operator who is manipulating the keyboard of an operation panel 41 and stored in the memory unit 38 under the control of the CPU 37.

For convenience of description, an example will now be described where T_4 (thyroxine) and TSH (thyroid stimulating hormone) in a blood serum are measured. These analysis items are displayed on the screen of a CRT 42 and selected. Reaction containers 2 storing beads coated with antibodies needed for respective analysis items are set on the reaction disk 1. For reference sample measurement and reagent blank, reaction containers devoid of bead are set. The correspondence between each of the reaction containers arrayed in sequence on the reaction disk and each of the analysis item, reference sample and reagent blank is set up and stored in the controller. Also, standard curves or working curves corresponding to respective analysis items are determined precedently and stored in the controller. Fig. 3 shows an example of a standard curve for T_4 and Fig. 4 an example for TSH.

The operation of the analyzer shown in Fig. 2 will now be described with reference to Fig. 1.

In step 101, the operator pushes the start key on the operation panel 41 of the analyzer to start analysis operation. In the analysis apparatus, the arm 14 a of the pipetting mechanism is first operated to pipette a predetermined amount of quinine sulfate solution, standing for the fluorescent reference sample, from a reagent cup 12 containing the quinine sulfate into the first reaction container (step 102). Thereafter, operation for pipetting a general test sample from a sample cup 11 corresponding to the following reaction container 2 is started (step 103).

The pipetting mechanism is controlled by the controller such that neither a latently fluorescent reagent containing a substrate nor an immunoreaction start reagent is added to the reaction container filled with the quinine sulfate. When this reaction container containing the reference sample reaches the photometry position 20, fluorescence stemming from the reference sample is measured by means of the fluorophotometer 19 (step 104). The photometry uses an exciting wavelength of 380 nm to measure a fluorescent wavelength of 460nm. The same exciting wavelength as above is applied to any analysis items of general test sample.

In step 105, the controller decides whether a measured value of the intensity of fluorescence stemming from the reference sample exceeds a predetermined setting value. If the results of comparison indicate that the measured value of the fluorescence emitted from the reference sample is less than the setting value, the procedure proceeds to step 106. At that time, the controller issues a command for stopping operation to individual mechanisms including the pipetting mechanism 33 and reaction disk 1 and as a result the analysis operation of the analysis apparatus is discontinued (step 107) and at the same time an alarm indicative of unsuitability of the optical system for the analysis of small amounts is displayed on the screen of the CRT 42 (step 108). In this case, the operation of the analysis apparatus once ends (step 109). Observing the alarm display, the operator checks the fluorophotometer 19 and exchanges the light source lamp or the photoelectric detectors, if degraded, with new ones to recover the performance of the analyzer.

If the results of comparison in step 105 indicate that the intensity of fluorescence stemming from the reference sample is greater than the setting value, the pipetting mechanism 33 continues pipetting general test samples (step 110). No sample is pipetted in a reaction container for blank measurement but a latently fluorescent reagent is added to the blank measurement reaction container by the same amount as that added to the general test sample. A sample solution is pipetted, by an amount of 50 μ l, from a sample cup 11 to a reaction container for general test sample and thereafter an immunoreaction reagent is 200 μ l pipetted in that reaction container (step 111). For example, as the immunoreaction reagent, a solution containing an antibody for each analysis item labeled by enzyme is used.

In the reaction container 2 on the reaction disk 1, an antigen/antibody reaction proceeds under the condition of heat insulation at 37°C to create antigen/antibody reaction complexes on a bead. The reaction container reaches the region of the washer 25 thirty minutes after start of the reaction and at the region, part of the solution containing substances which have not been reacted is discharged from the reaction

container, a cleansing solution is 500 μ l charged into the reaction container and the used cleansing solution is finally discharged from the reaction container (step 112). The above bead washing operation using the cleansing solution is repeated three times.

On the other hand, the pipetting mechanism 14 operates to pipette the reference sample solution and the latently fluorescent reagent into corresponding reaction containers for reference sample and reagent blank which are disposed at predetermined intervals in the train of reaction containers (step 113).

The pipetting mechanism 14 then pipettes a latently fluorescent reagent into the reaction container for general test sample washed in the step 112. As the latently fluorescent reagent, a substrate and a buffer solution are added by 50 μ l and 200 μ l, respectively, (step 114) and an enzyme reaction proceeds for 30 minutes at 37° C. In this example, a solution of 4-methylumbelliferyl phosphate is used as the substrate and alkali phosphatase is used as the enzyme to proceed with the enzyme reaction. Through this reaction, 4-methylumbelliferone is created which is a fluorescent substance.

In step 115, it is decided whether the reaction container positioned at the photometry position 20 is for the reference sample and similarly, it is decided in step 116 whether the reaction container reaching the photometry position is for the reagent blank. For the reaction solution of general test sample, the intensity of fluorescence stemming therefrom is measured 15 minutes after the step 114 (step 117). The amount of 4-methylumbelliferone created in the reaction solution depends on the amount of antigen/antibody reaction complexes on the bead.

When the reaction container for reference sample reaches the photometry position 20, the procedure branches from step 115 to step 122, at which the intensity of fluorescence stemming from the quinine sulfate solution is measured. A correction coefficient for a standard curve stored in advance is calculated on the basis of a measured value of the intensity of fluorescence stemming from the reference sample (step 123) and used for correcting the standard curve (step 124).

When the reaction container for reagent blank reaches the photometry position 20, the procedure branches from step 116 to step 118, at which the intensity of fluorescence stemming from the reagent blank that is unaffected by the antigen/antibody reaction complex is measured, and the fluorescence intensity is decided as to whether to be greater than an acceptable value stored in advance (step 119). If a value measured for the reagent blank exceeds the acceptable value, an alarm indicative of degradation of the latently fluorescent reagent is displayed on the screen of the CRT 42 (step 120). This alarm is printed out of a printer 43 upon delivery of results of analysis in step 126.

If the value measured for reagent blank is less than the acceptable value, the fluorophotometry of the reaction solution of general test sample follows and the measured value of the intensity of fluorescence stemming from the reaction solution is corrected on the basis of a reagent blank value (step 121) and then used for being compared with the standard curve. Even if the reagent blank value is decided to be greater than the acceptable value, the analysis apparatus may be allowed not to be discontinued but to continue analyzing general test samples, provided that the operator fulfils exchange of the substrate solution in accordance with the alarm display.

In step 125, element concentrating corresponding to the measured values for the general test samples are calculated from the standard curve corrected on the basis of the measured value for the reference sample and the measured value for reagent blank, and in step 126 analysis values of individual test samples are delivered. In this example, the ratio between a photometrical value measured for the reference sample when preparing a standard curve and a photometrical value measured for the reference sample immediately before conducting photometry of the test sample is used to correct the standard curve itself but alternatively a value measured for the test sample may first be corrected by the correction coefficient and then applied to the initial standard curve for the purpose of calculating concentration of analysis elements. When the analysis operations for all test samples are completed, operation of individual mechanisms of the analysis apparatus ends (step 127).

50 Experimental Example

The same control blood serum was pipetted into 20 reaction containers and T4 and TSH were measured in accordance with the flow shown in Fig. 1. Results of measurement are shown in Table 1. Net data, representative of measured values before correction, for individual test samples is suffixed with A and data after correction is suffixed with B. The reference sample was quinine sulfate solution, and a photometrical value measured for the quinine when experimentally obtaining data in Table 1 was 170.3 and a photometrical value measured for the quinine when preparing the standard curve was 165.4. Accordingly, the correction coefficient is 165.4/170.3.

Table 1

Sample	T ₄ (A)	T ₄ (B)	TSH(A)	TSH(B)
No. 1	403.2	391.6	262.0	254.5
2	402.8	391.2	261.1	253.6
3	404.5	392.9	263.3	255.7
4	401.0	389.5	261.7	254.1
5	402.9	391.3	262.5	254.9
6	403.1	391.5	262.4	254.9
7	401.6	390.0	261.3	254.3
8	403.7	392.1	261.9	254.4
9	404.1	392.5	263.4	255.8
10	404.3	392.7	260.6	253.1
11	405.8	394.1	261.8	254.3
12	400.9	389.4	262.9	255.3
13	401.3	389.8	263.7	256.1
14	403.2	391.6	260.4	252.9
15	404.6	393.3	261.7	254.2

Sample	T ₄ (A)	T ₄ (B)	TSH(A)	TSH(B)
16	400.2	388.7	260.2	252.7
17	402.5	390.9	262.4	254.9
18	403.4	391.8	264.0	256.4
19	401.4	389.9	263.1	255.5
20	404.2	392.6	263.4	255.8

After data in Table 1 was measured, the same control blood serum was pipetted into 10 reaction containers and subjected to analysis measurement similarly to the precedence. Net data for individual test samples thus obtained is suffixed with A' and data after correction is suffixed with B'. In this measurement, a photometrical value for the reference sample was 175.6 and results of the measurement are shown in Table 2. Since the photometrical value measured for the quinine sulfate solution when preparing the standard curve is 165.4, the correction coefficient used for obtaining data in Table 2 is 165.4/175.6.

Table 2

Sample No.	T ₄ (A')	T ₄ (B')	TSH(A')	TSH(B')
1	423.5	398.9	268.8	253.2
2	415.4	391.3	269.7	254.0
3	416.7	392.5	269.2	253.6
4	415.7	391.6	269.9	254.2
5	418.8	394.5	271.7	255.9
6	414.9	390.8	269.5	253.8
7	416.8	392.6	270.4	254.7
8	415.4	391.3	271.3	255.5

Sample No.	T ₄ (A')	T ₄ (B')	TSH(A')	TSH(B')
9	416.3	392.1	268.7	253.1
10	416.2	392.0	270.2	254.5

For T₄, an average value \bar{B} of the corrected values in Table 1 is 391.4 and an average value \bar{B} of the corrected values in Table 2 is 391.9, demonstrating that even when the measurement is not carried out on the same line, the influence of drift of the analysis apparatus can be removed to ensure highly reproducible measurement.

A second embodiment of the invention will now be described. In this embodiment, the immunoanalysis apparatus shown in Fig. 2 is also used. As the reference sample, a rhodamine B solution is used and a cup containing the reference sample is disposed on the reagent disk 9. The analyzer is operated in accordance with a flow similar to that shown in Fig. 1 to measure T₄ and TSH. No latently fluorescent reagent is added to the rhodamine B solution and the intensity of fluorescent stemming from the rhodamine B itself is measured. In this embodiment, the wavelength of exciting light for the reference sample is 550 nm and the wavelength of fluorescence excited from the reference sample is 590 nm.

In connection with the second embodiment, reproducibility of measurement was examined similarly to the previous embodiment. Ten samples of the same control blood serum were measured twice at different times. The value of fluorescence intensity measured for the reference sample of rhodamine B when preparing the standard curve was 451.7, measured for the reference sample when conducting the first measurement of the control blood serum was 438.2 and measured for the reference sample when conducting the second measurement of the control blood serum was 420.8. When measured values for test samples are corrected using the measured value for reference sample and the measured value for reagent blank, an average value of measured values in the first measurement was 382.1 and an average value of measured values in the second measurement was 382.7. Thus, even with rhodamine B used as the reference sample, the influence of drift can be removed and the difference between batches can be excluded.

A third embodiment of the invention will now be described. In this embodiment, the immunoanalysis apparatus shown in Fig. 2 is also used. As the reference sample, water is employed in this embodiment. The analysis apparatus is operated in accordance with a flow similar to that shown in Fig. 1 to measure T₄ and TSH. In this embodiment, addition of any latently fluorescent reagent is not effected, either, and the intensity of fluorescence stemming from water itself is measured. The wavelength of exciting light for the water serving as the reference sample is 380 nm and the wavelength of fluorescence excited from the reference sample is 470 nm.

In connection with the third embodiment, reproducibility of measurement was also examined. Ten samples of the same control blood serum were measured twice at different times. The value of fluorescence intensity measured for the reference sample of water when preparing the standard curve was 95.56, measured for the reference sample when conducting the first measurement of the control blood serum was 93.24 and measured for the reference sample when conducting the second measurement was 91.73. When measured values for test samples are corrected using the measured values for reference sample, an average value for T₄ was 393.0 in the first measurement and 391.0 in the second embodiment and an average value for TSH was 289.7 in the first measurement and 289.6 in the second embodiment.

A fourth embodiment of the invention will now be described. In this embodiment, not the substrate but liposomes sensitized by antibodies are used as the latently fluorescent reagent. This embodiment will be explained by referring to, for example, TSH as a measurement item. The analysis apparatus shown in Fig. 2 is also used and a reference sample of quinine sulfate and a solution of dispersed liposomes each having sealed-in FITC serving as fluorescence marker are set on the reagent disk 9. Anti-TSH antibodies are attached to the surface of the liposome. As shown in Fig. 5, the same anti-TSH antibodies 51 as above are attached to the surface of a bead contained in a reaction container 2. Ten samples of control blood serum are set on the sample disk 8. An example of a standard curve prepared in advance is shown in Fig. 6.

In this embodiment, the operation of the analysis apparatus also follows the steps 101 to 113 in Fig. 1. In step 105, the value measured for the reference sample of quinine sulfate is decided to be greater than the setting value. When the control blood serum is added in the reaction container 2, antigens 53 (TSH) in the blood serum sample couple to the antibodies 51 on the bead 50 to create antigen/antibody reaction complexes 54. Thereafter, a reagent containing liposomes 55 sensitized by antibodies is added.

Thirty minutes after start of an immunoreaction, the washer 25 operates to remove part of reagent and part of sample which have not been reacted in the reaction container and after washing, a buffer solution containing a surfactant is 500 μ l added in the reaction container. This ruptures the enclosure of the liposome 55 and the sealed-in FITC flows out. The reaction container containing this reaction solution is positioned at the photometry position 20 and fluorophotometry is then carried out.

The value measured for test samples is corrected using the measured values for the reference sample and reagent blank in a way shown in the flow chart of Fig. 1. The correction coefficient for standard curve based on the fluorophotometry of the reference sample was 165.4/175.4. The average value of fluorescence intensity of measured values for 10 analyzed TSH samples was 270.2 and the corrected average value was 254.8.

35 Claims

1. A fluorescence analysis method in which a sample (11) to be tested and a latently fluorescent reagent (12) are pipetted into a reaction container (2) and a reaction solution containing a fluorescent substance created in the reaction container is measured by means of a fluorophotometer (19), said method comprising the steps of:

delivering (102) a reference sample (12) which is able to fluoresce to a reaction container by means of pipetting means (15) prior to measurement of said reaction solution;
measuring (104) the intensity of fluorescence stemming from said reference sample by means of said fluorophotometer;
comparing (105) a predetermined setting value with a value measured for said reference sample; and causing said pipetting means to discontinue (106) the subsequent sampling operation when the results of comparison indicate that said measured value is less than said setting value but to continue (110) the subsequent sampling operation when the results of comparison indicate that said measured value is greater than said setting value.

2. A fluorescence analysis method according to Claim 1 wherein said reference sample is a solution containing a substance which is able to fluoresce by itself.

3. A fluorescence analysis method according to Claim 2 wherein said substance capable of fluorescing is selected from the group consisting of quinine sulfate, rhodamine B and water.

4. A fluorescence analysis method according to Claim 1 wherein said latently fluorescent reagent comprises a substrate which can turn into a fluorescent substance.

5. A fluorescence analysis method according to Claim 1 wherein said latently fluorescent reagent comprises a liposome incorporating a fluorescent substance.

6. A fluorescence analysis method according to Claim 1 which proceeds with, when said sampling

operation is continued, the steps of:

measuring (118) the intensity of fluorescence stemming from a blank solution in a reaction container to produce a blank measured value in mid course of measurement of a plurality of test samples, said blank solution not containing any test sample but containing a latently fluorescent reagent; and

5 delivering (120) an alarm when said blank measured value is greater than a predetermined acceptable upper limit.

7. A fluorescence analysis method according to Claim 1 which proceeds with, when said sampling operation is continued, the steps of:

10 producing (117) a first measured value of fluorescence emitted from said test sample by measuring the intensity of fluorescence stemming from a fluorescent substance created in said reaction container containing the test sample and latently fluorescent reagent;

producing (122) a second measured value of fluorescence emitted from said reference sample by measuring the intensity of fluorescence stemming from said reference sample in mid course of measurement of a plurality of test samples; and

15 correcting said first fluorescence measured value on the basis of said second fluorescence measured value.

8. A fluorescence analysis method according to Claim 1 which proceeds with, when said sampling operation is continued, the steps of:

20 creating an immunocomplex (54) of an antibody (or antigen) (51) attached to a solid phase (50) and an antigen (or antibody) (53) contained in a test sample, within a reaction container and labeling immunocomplex by enzyme;

creating a fluorescent substance by adding a substrate in said reaction container containing said immunocomplex; and

measuring the intensity of fluorescence stemming from a reaction solution created from said fluorescent substance by means of said fluorophotometer.

25 9. An automatic fluorescence analyzer comprising:

reaction disk means (1) for holding and moving a plurality of reaction containers (2);

pipetting means (15) for delivering test samples (11) and/or reagents (12) to said reaction containers;

fluorophotometric means (19) for irradiating exciting light on a reaction container held on said reaction disk and measuring fluorescence emitted from a solution in said reaction container;

30 control means having a memory unit (38) and being operative to control operations of organization elements in said analyzer; and

input means (41) for entering data into said memory unit;

said control means being operable to store a setting value inputted from said input means in said memory unit and operable to compare a measured value obtained through photometry of a reference sample with

35 said setting value stored in said memory unit and discontinue the subsequent operation of said pipetting means when the results of comparison indicate that said measured value is less than said setting value.

10. An automatic fluorescence analyzer according to Claim 9 further comprising output means (42) for delivering the results of measurement, wherein said control means operates to compare a blank measured value, obtained through measurement of a reagent blank solution not containing any test sample but
40 containing a reagent, with a predetermined acceptable upper limit and deliver an alarm to said output means when the results of comparison indicate that said blank measured value is greater than said acceptable upper limit.

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FIG. 1

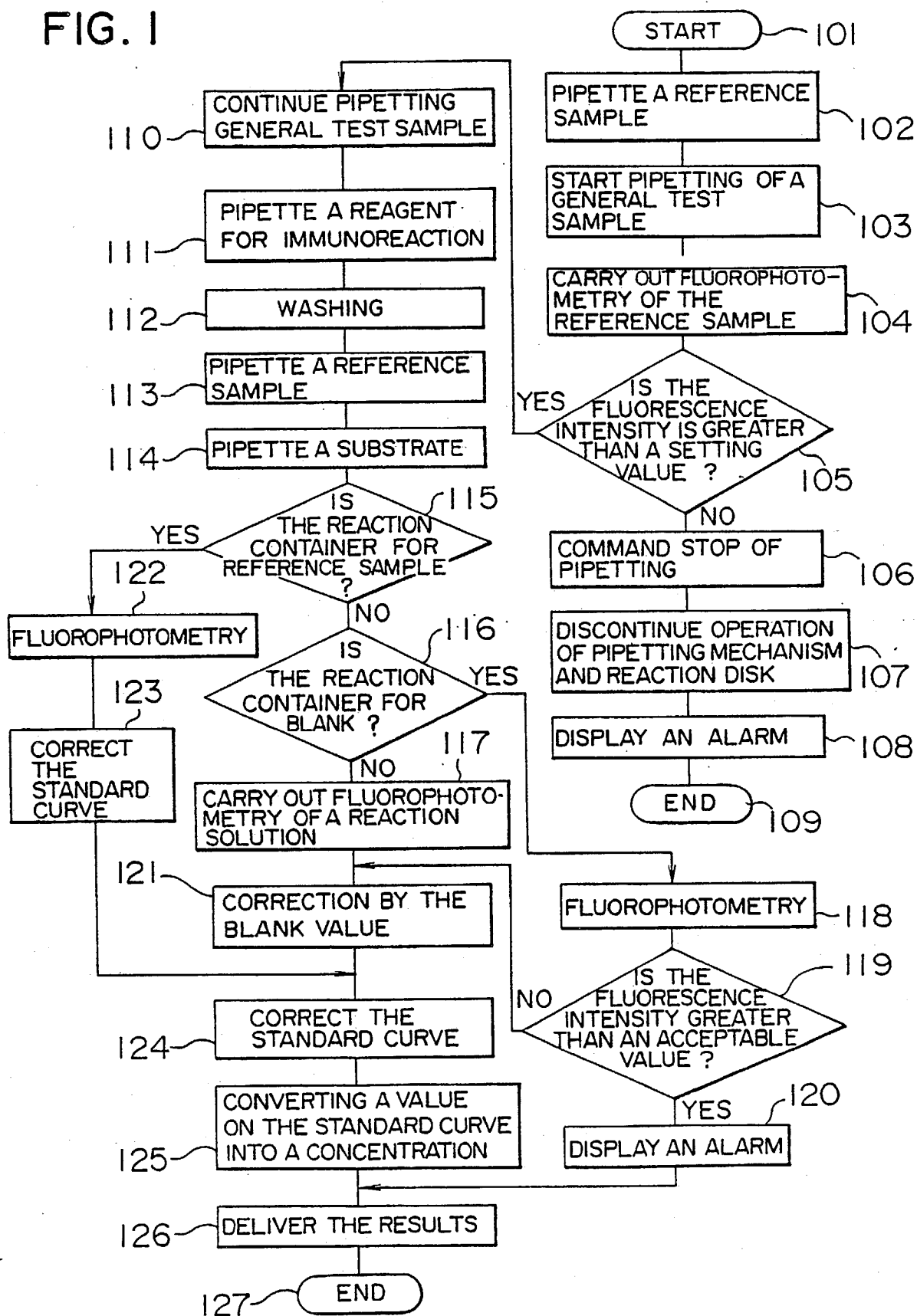


FIG. 2

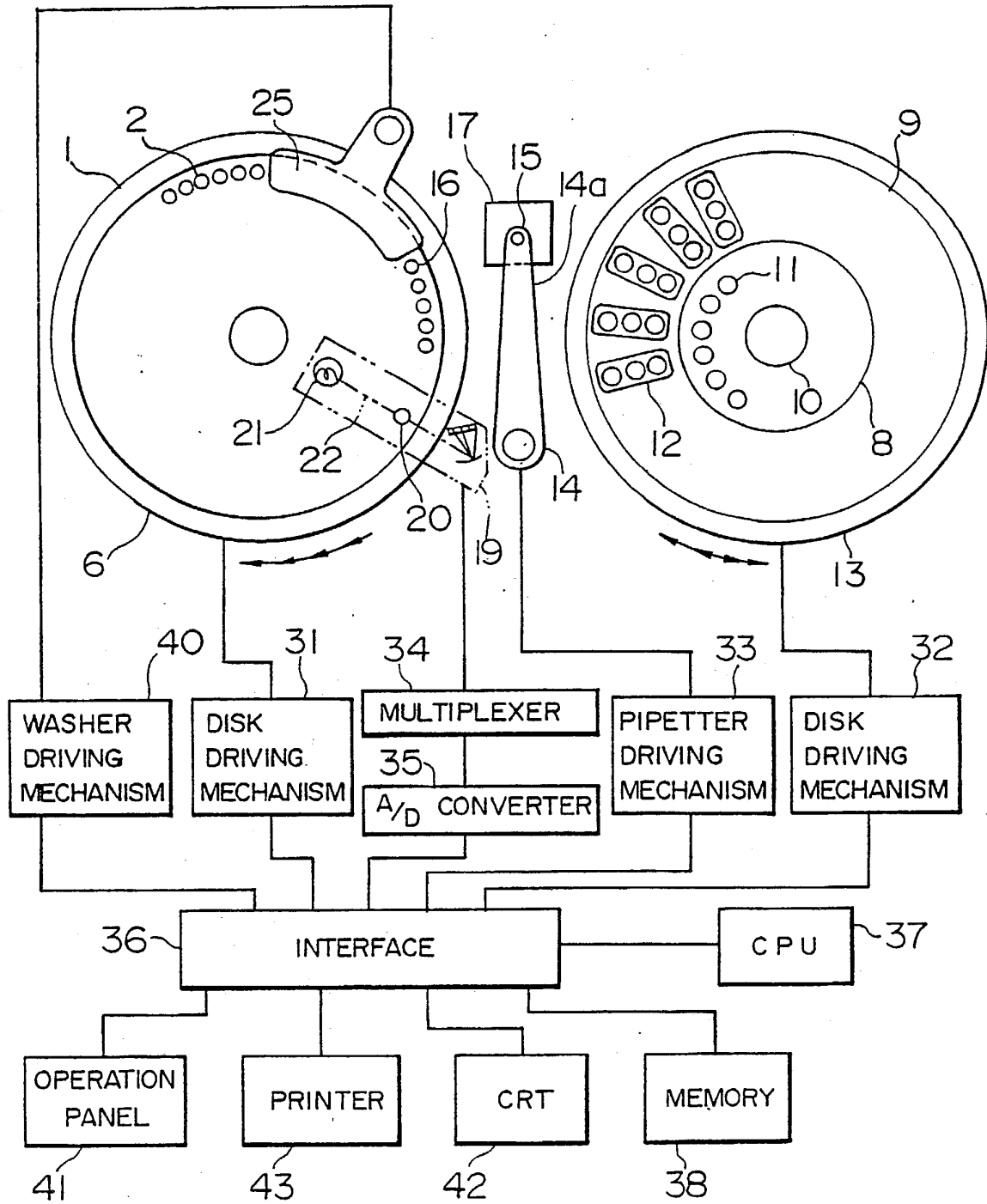


FIG. 3

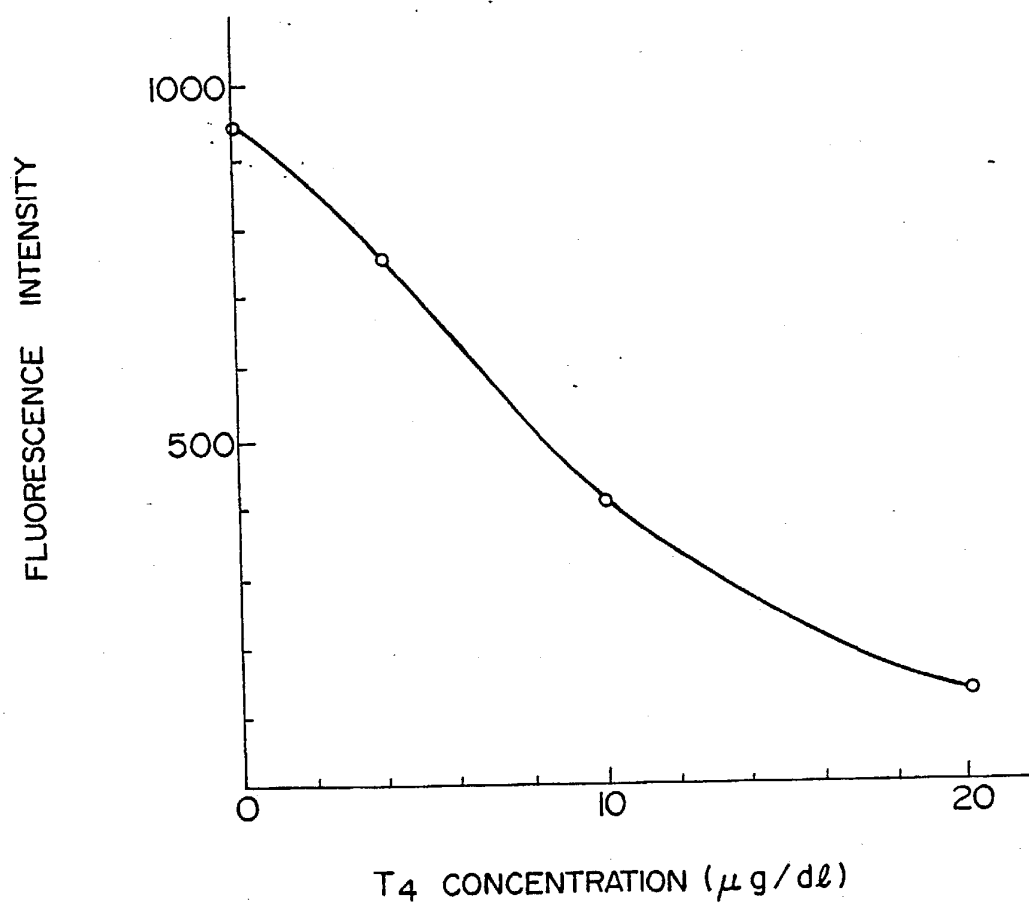


FIG. 4

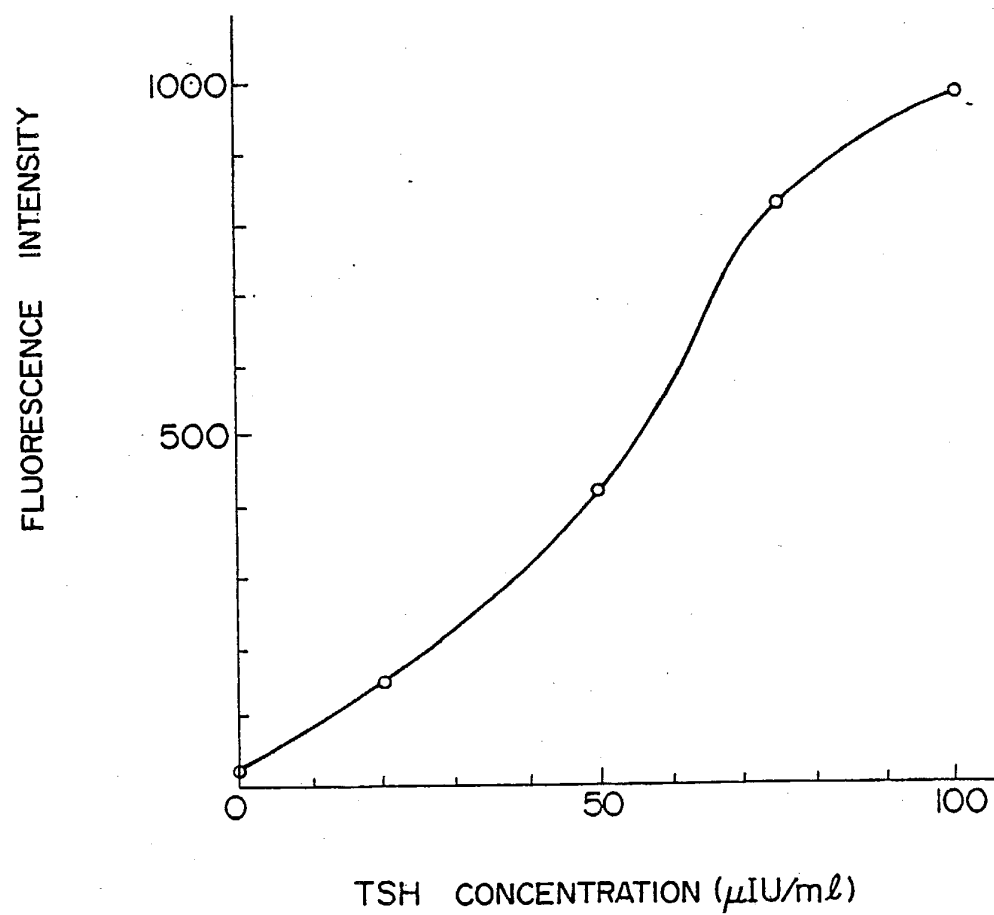
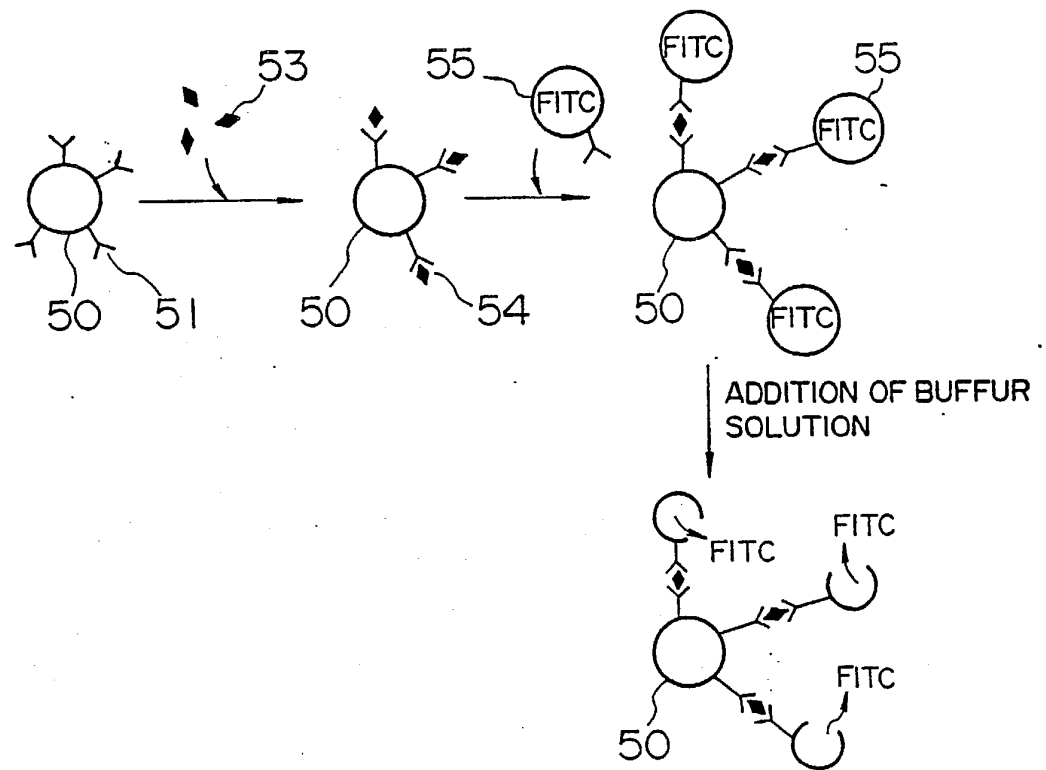
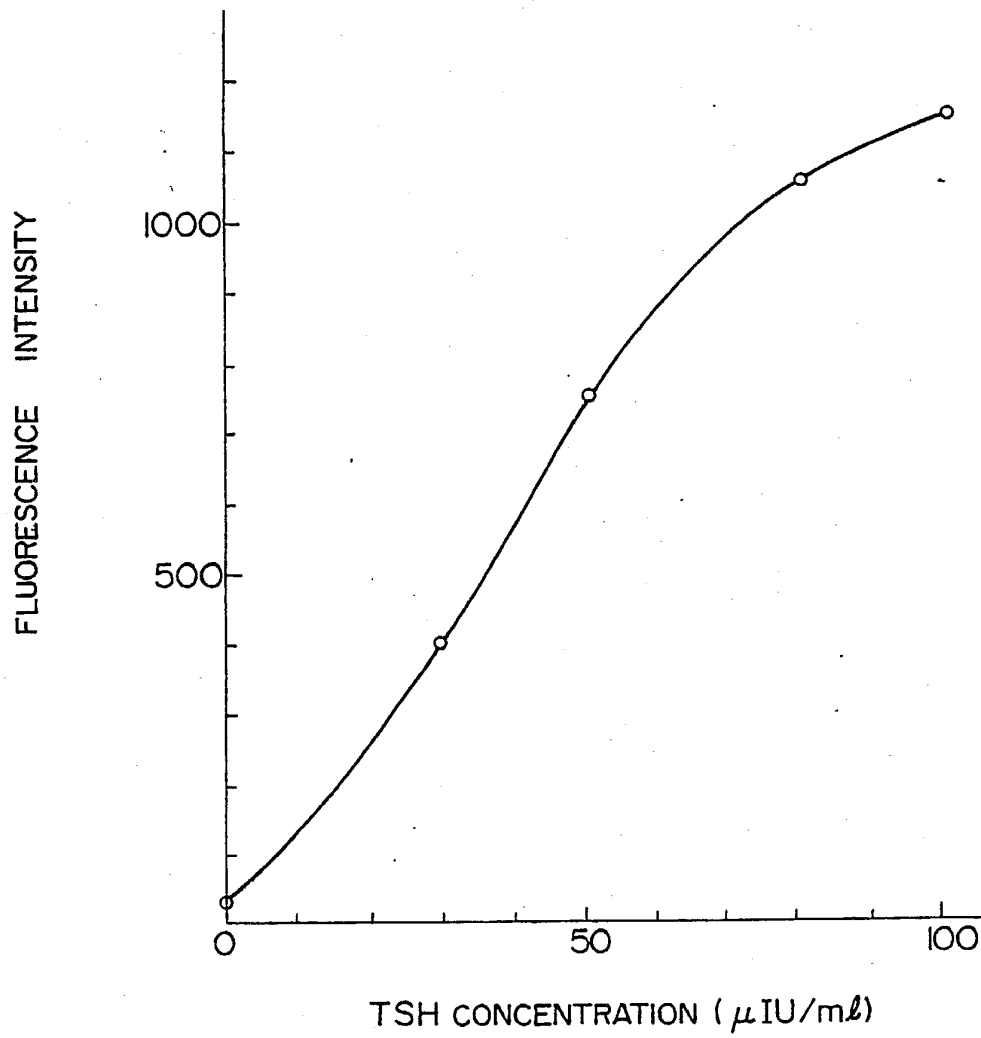


FIG. 5



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FIG. 6



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(11) Publication number:

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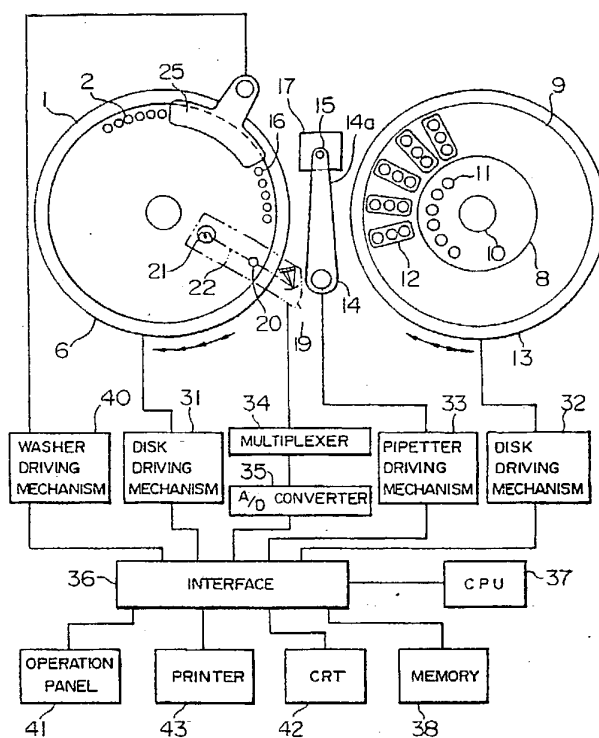
EUROPEAN PATENT APPLICATION(21) Application number: **89115739.8**(51) Int. Cl.⁵: **G01N 21/64, G01N 35/02,
G01N 33/543**(22) Date of filing: **25.08.89**(30) Priority: **26.08.88 JP 210741/88**(43) Date of publication of application:
28.02.90 Bulletin 90/09(84) Designated Contracting States:
DE FR GB(68) Date of deferred publication of the search report:
03.07.91 Bulletin 91/27(71) Applicant: **HITACHI, LTD.**
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W-8000 München 86(DE)(54) **Method and apparatus for automatic measurement of fluorescence.**

(57) An analyzer has a reaction disk (1) for holding a plurality of reaction containers (2) and a fluorophotometer (19) for measuring fluorescence stemming from solutions in the containers. Most of the containers contain solid phases (50) attached with antibodies (51) but at least one container does not contain any solid phase. In normal operation of the analyzer, a test sample (11) containing antigens (53) and a latently fluorescent reagent such as antibodies labeled by enzyme are added to a container (2) containing a solid phase (50). In this container, a fluorescent substance is created through an enzyme reaction. Exciting light (22) is irradiated on the container and fluorescence emitted from the fluorescent substance is measured. In mid course of measure-

ment operation of test samples, fluorescence stemming from a reference sample (12) such as quinine sulfate is measured to produce measured values for the reference sample by which measured values for the test samples are corrected. Prior to measurement of general test samples (11) by the analyzer, fluorescence stemming from a reference sample (12) in a container removed of any solid phase is measured to enable the analyzer itself to decide whether the analyzer operates normally. In this case, a value measured for the reference sample is compared with a setting value. When the results of comparison indicate that the normal operation is difficult to continue, the sampling operation by the pipetter is discontinued.

EP 0 355 849 A3

FIG. 2





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EUROPEAN SEARCH REPORT

Application Number

EP 89 11 5739

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	US-A-4 536 655 (BARNES) * Column 4, lines 26-41; claim 12 * -----	1,9	G 01 N 21/64 G 01 N 35/02 G 01 N 33/543
A	FR-A-1 588 298 (RAIT) * Page 9, line 41 * -----	1	
A	US-A-3 973 129 (BLUMBERG) * Column 5, lines 47-61 * -----	1,2	
A,D	PATENT ABSTRACTS OF JAPAN, vol. 11, no. 239 (P-602)[2686], 6th August 1987; & JP-A-62 50 662 (TOYO SODA) 05-03-1987 * Abstract * -----	8	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			G 01 N
The present search report has been drawn up for all claims			
Place of search		Date of completion of search	Examiner
The Hague		20 March 91	KRAMETZ E.M.
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